

**CLAIMS**

1. A coronavirus Spike (S) protein, or fragment thereof, having at least 75% amino acid sequence identity with the CRCV S protein whose amino acid sequence is listed in Figure 4, and comprising at least one of the canine respiratory coronavirus (CRCV)-specific amino acids listed in Table 1.  
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2. A coronavirus S protein that comprises the amino acid sequence listed in Figure 4, or a variant thereof with at least 97% amino acid sequence identity with the sequence listed in Figure 4.  
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3. A coronavirus polymerase (pol) protein, or fragment thereof, having at least 90% amino acid sequence identity with BCV pol protein and comprising the amino acid E at the position corresponding to position 4975 in the BCV genome (Accession No. SWALL: Q91A29).  
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4. A coronavirus pol protein that comprises the amino acid sequence listed in Figure 2.
- 20 5. A coronavirus hemagglutinin/esterase (HE) protein, or fragment thereof, having at least 90% amino acid sequence identity with BCV HE protein and comprising one or more of F at position 235, N at position 242 and L at position 253 of the BCV HE protein (Genbank Accession No. AF058942).  
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6. A coronavirus HE protein that comprises the amino acid sequence listed in Figure 14.

7. A polynucleotide that encodes the protein according to any of Claims 1 to 6, or the complement thereof.
8. A polynucleotide according to Claim 7 comprising the nucleotide  
5 sequence listed in Figure 3.
9. A polynucleotide according to Claim 7 comprising the nucleotide sequence listed in Figure 1.
10. A polynucleotide according to Claim 7 comprising the nucleotide  
10 sequence listed in Figure 13.
11. A vector comprising the polynucleotide of any of Claims 7 to 10.
12. A host cell comprising the vector of Claim 11.  
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13. A method of obtaining a protein encoded by the vector of Claim 11, the method comprising culturing the host cell of Claim 12, expressing the protein in the host cell, and purifying the protein.  
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14. A protein obtainable by the method of Claim 13.
15. A host cell according to Claim 12 wherein the vector is an expression vector comprising a eukaryotic promoter operatively linked to the  
25 polynucleotide, and wherein the host cell is a eukaryotic host cell.
16. A method of obtaining a glycosylated S protein encoded by the polynucleotide of Claim 7 or 8, the method comprising culturing the host cell of Claim 15, expressing the protein in the host cell, and purifying the  
30 protein.

17. A glycosylated S protein obtainable by the method of Claim 16.
18. A method of making an anti-CRCV antibody comprising (i) raising  
5 an immune response to an S protein according to any of Claims 1, 2, 14 or  
17 in an animal and preparing an antibody from the animal or from an  
immortal cell derived therefrom, or (ii) selecting an antibody from an  
antibody-display library using an S protein according to any of Claims 1, 2,  
14 or 17.
- 10 19. A method according to Claim 18 further comprising determining  
whether the antibody has greater affinity for the CRCV S protein than for  
the BCV S protein.
- 15 20. An anti-CRCV antibody obtainable by the method of Claim 18 or 19  
, that has greater affinity for the CRCV S protein than for the BCV S  
protein.
21. A method of making an anti-CRCV antibody comprising (i) raising  
20 an immune response to an HE protein according to Claim 5 or 6 in an  
animal and preparing an antibody from the animal or from an immortal cell  
derived therefrom, or (ii) selecting an antibody from an antibody-display  
library using an HE protein according to Claim 5 or 6.
- 25 22. A method according to Claim 21 further comprising determining  
whether the antibody has greater affinity for the CRCV HE protein than for  
the BCV HE protein.

23. An anti-CRCV antibody obtainable by the method of Claim 21 or 22, that has greater affinity for the CRCV HE protein than for the BCV HE protein.
- 5 24. A method of determining whether a dog has been exposed to CRCV, the method comprising:
- (a) obtaining a suitable sample from the dog; and
  - (b) identifying CRCV or an anti-CRCV antibody in the sample.
- 10 25. A method according to Claim 24 or 25 wherein the anti-CRCV antibody is detected using a CRCV, BCV, human coronavirus (HCV) or hemagglutinating encephalomyelitis virus (HEV) antigen.
26. A method according to Claim 25 wherein the suitable sample is an  
15 antibody containing sample such as serum, saliva, tracheal wash and bronchiolar lavage, and wherein identifying an anti-CRCV antibody comprises identifying an antibody that selectively binds to BCV S protein (AF058942) HCV S protein (L14643), or to a coronavirus having an S protein with at least 75% amino acid identity with CRCV S protein (Figure  
20 4) or a fragment thereof.
27. A method according to Claim 25 wherein identifying an anti-CRCV antibody comprises identifying an antibody that selectively binds to BCV HE protein (AF058942) HCV HE protein (M76373), or to a coronavirus  
25 having an HE protein with at least 90% amino acid identity with CRCV HE protein (Figure 14) or a fragment thereof.
28. A method according to Claim 24 wherein the suitable sample is an antibody containing sample, and wherein identifying an anti-CRCV  
30 antibody comprises identifying an antibody that selectively binds to an S

protein according to any of Claims 1, 2, 11 or 14 or to an HE protein according to Claim 5 or 6.

29. A method according to Claim 24 wherein the suitable sample is a  
5 lung wash, tracheal wash, tonsillar swab or a biopsy or post-mortem sample from the respiratory tract of the dog.

30. A method according to Claim 29 wherein identifying CRCV comprises identifying a nucleic acid component of CRCV.

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31. A method according to Claim 30 wherein identifying a nucleic acid component of CRCV comprises identifying a polynucleotide that hybridises at high stringency to the BCV genome (AF058942).

15 32. A method according to Claim 30 or 31 wherein identifying CRCV comprises identifying a polynucleotide as defined in any of Claims 7 to 10.

33. A method according to Claim 29 wherein identifying CRCV comprises identifying a protein component of CRCV.

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34. A method according to Claim 33 wherein identifying a protein component of CRCV comprises identifying a protein according to any of Claims 1, 2, 5, 6, 11 or 14.

25 35. A method according to Claim 33 or 34 wherein identifying a protein component of CRCV comprises using an antibody reactive with CRCV.

36. A method according to Claim 35 wherein the antibody reactive with CRCV is an anti-BCV antibody, an anti-HCV antibody, or an anti-CRCV  
30 antibody according to Claim 20 or 23.

37. An immunosorbent assay for detecting anti-CRCV S antibodies, the assay comprising:

a solid phase coated with an S protein according to any of Claims 1,  
5 2, 14 or 17, or an antigenic fragment thereof, wherein anti-CRCV S  
antibodies in a sample exposed to the solid phase will bind to the protein;  
and

a detectable label conjugate which will bind to the anti-CRCV  
antibodies bound to the solid phase.

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38. An immunosorbent assay for detecting anti-CRCV HE antibodies,  
the assay comprising:

a solid phase coated with an HE protein according to Claim 5 or 6, or  
an antigenic fragment thereof, wherein anti-CRCV HE antibodies in a  
15 sample exposed to the solid phase will bind to the protein; and

a detectable label conjugate which will bind to the anti-CRCV  
antibodies bound to the solid phase.

39. An immunosorbent assay according to Claim 37 or 38, wherein the  
20 solid phase is a microtitre well.

40. An immunosorbent assay according to Claim 37 or 38, wherein the  
conjugate comprises anti-dog antibody.

25 41. An immunosorbent assay according to any of Claims 37 to 40,  
wherein the conjugate comprises an enzyme.

42. An immunosorbent assay according to Claim 41, wherein the  
enzyme is horseradish peroxidase.

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43. An immunosorbent assay according to Claim 41 or 42, further comprising a substrate for the enzyme.
44. A solid substrate with an S protein according to any of Claims 1, 2, 14 or 17, or an antigenic fragment thereof, attached thereto, for capturing anti-CRCV S antibodies from a liquid sample, wherein anti-CRCV S antibodies in a sample exposed to the solid substrate will bind to the S protein.
45. A solid substrate with an HE protein according to Claim 5 or 6, or an antigenic fragment thereof, attached thereto, for capturing anti-CRCV HE antibodies from a liquid sample, wherein anti-CRCV HE antibodies in a sample exposed to the solid substrate will bind to the HE protein.
46. A solid substrate according to Claim 44 or 45, wherein the solid substrate is a microtitre well.
47. A vaccine composition for vaccinating dogs comprising a coronavirus having an S protein with at 75% least amino acid identity with BCV S protein, or a coronaviral protein having at least 75% amino acid identity with a BCV protein or an immunogenic fragment thereof, or a nucleic acid encoding said coronaviral protein or immunogenic fraction thereof.
48. A vaccine composition according to Claim 47 wherein the coronaviral protein is a BCV, HCV, HEV or CRCV protein, or a modification thereof.
49. A vaccine composition according to Claim 47 or 48 wherein the coronaviral protein is an S protein.

50. A vaccine composition according to Claim 49 comprising an S protein according to any of Claims 1, 2, 14 or 17, a BCV S protein, an HCV S protein, an HEV S protein, or an immunogenic fragment thereof.
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51. A vaccine composition according to Claim 47 or 48 wherein the coronaviral protein is a hemagglutinin-esterase protein (HE) or an integral membrane protein (M).
- 10 52. A vaccine composition according to Claim 51 wherein the HE protein comprises an HE protein as defined in Claim 5 or 6, a BCV HE protein, an HCV HE protein, or an immunogenic fragment thereof.
53. A vaccine composition according to Claim 47 wherein the virus is  
15 selected from BCV, HCV, HEV and CRCV, or a modification thereof.
54. A vaccine composition according to any of Claims 47 to 53 and also comprising a pharmaceutically acceptable adjuvant.
- 20 55. A vaccine composition according to any of Claims 47 to 55 further comprising any one or more of:
- (a) an agent capable of raising an immune response in a dog against canine parainfluenza virus (CPIV);
  - (b) an agent capable of raising an immune response in a dog  
25 against canine adenovirus type 2 (CAV-2);
  - (c) an agent capable of raising an immune response in a dog against canine herpesvirus (CHV); and
  - (d) an agent capable of raising an immune response in a dog against *Bordetella bronchiseptica* (*B. bronchiseptica*).



56. Use of (i) a coronavirus having an S protein with at least 75% amino acid identity with CRCV S protein, or (ii) a coronavirus having an S protein with at least 75% amino acid identity with BCV S protein, or (iii) a coronavirus having an HE protein with at least 90% amino acid identity with CRCV HE protein, or (iv) a coronavirus having an HE protein with at least 90% amino acid identity with BCV HE protein, or (v) a coronavirus protein having at least 75% amino acid identity with a CRCV protein or an immunogenic fragment thereof, or (vi) a coronaviral protein having at least 75% amino acid identity with a BCV protein, or an immunogenic fragment thereof, or (vii) a nucleic acid encoding said coronaviral protein or immunogenic fraction thereof, in the preparation of a medicament for stimulating an immune response against CRCV in a dog.

57. Use of (i) a coronavirus having an S protein with at least 75% amino acid identity with CRCV S protein, or (ii) a coronavirus having an S protein with at least 75% amino acid identity with BCV S protein, or (iii) a coronavirus having an HE protein with at least 90% amino acid identity with CRCV HE protein, or (iv) a coronavirus having an HE protein with at least 90% amino acid identity with BCV HE protein, or (v) a coronavirus protein having at least 75% amino acid identity with a CRCV protein or an immunogenic fragment thereof, or (vi) a coronaviral protein having at least 75% amino acid identity with a BCV protein, or an immunogenic fragment thereof, or (vii) a nucleic acid encoding said coronaviral protein or immunogenic fraction thereof, in the preparation of a medicament for prophylaxis of respiratory disease in a dog.

58. Use according to Claim 56 or 57 wherein the coronaviral protein is a BCV, HCV, HEV or CRCV protein, or a modification thereof.

59. Use according to any of Claims 56 to 58 wherein the coronaviral protein is an S protein.

60. Use according to Claim 59 wherein the S protein comprises an S protein according to any of Claims 1, 2, 14 or 17, a BCV S protein, an HCV S protein, or an immunogenic fragment thereof.

61. Use according to any of Claims 56 to 58 wherein the coronaviral protein is HE or M.

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62. Use according to Claim 61 wherein the HE protein comprises an HE protein according to Claim 5 or 6, a BCV HE protein, an HCV HE protein, or an immunogenic fragment thereof.

15 63. Use according to Claim 56 wherein the virus is selected from BCV, HCV, HEV and CRCV, or a modification thereof.

64. An S protein according to any of Claims 1, 2, 14 or 17 for use in medicine.

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65. An HE protein according to Claim 5 or 6 for use in medicine.

66. A method of vaccinating a dog against CRCV, the method comprising administering to the dog a vaccine composition according to any of Claims 47 to 54.

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67. A method for combating the spread of CRCV between dogs comprising determining whether a dog is infected with CRCV according to the method of any of Claims 24 to 36 and, if the dog is infected with CRCV, quarantining the dog.

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68. A method for combating the spread of CRCV between dogs comprising determining whether a dog is infected with CRCV according to the method of any of Claims 24 to 36 and, if the dog is infected with CRCV,  
5 vaccinating other dogs that have been, are, or are likely to be in contact with the dog.

69. A method for identifying a test vaccine capable of preventing canine infectious respiratory disease (CIRD) in dogs, comprising  
10 (a) determining whether a dog has been exposed to CRCV according to the method of any of Claims 24 to 36,  
(b) if the dog has not been exposed to CRCV, administering the test vaccine to the dog,  
(c) inoculating the dog with CRCV, and  
15 (d) determining whether the dog develops CIRD,  
wherein the absence of CIRD in step (d) indicates that the test vaccine is capable of preventing CIRD.

70. A vaccine identified by the method of Claim 69.  
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71. The *E. coli* strain Spike D-1 CRCV, containing a plasmid whose insert contains a portion of the CRCV S cDNA, as deposited by the Royal Veterinary College at the NCIMB under Accession number NCIMB 41146 on 25 July 2002.

25 72. The plasmid contained in *E. coli* strain Spike D-1 CRCV, deposited by the Royal Veterinary College at the NCIMB under Accession number NCIMB 41146 on 25 July 2002.

73. A kit of parts for the immunosorbent assay according to any of Claims 37-43, comprising a solid phase, a CRCV or CRCV-like S protein and/or a CRCV or CRCV-like HE protein for coating the solid phase, and a detectable label conjugate.

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74. A kit of parts according to Claim 73 wherein the solid phase comprises a microtitre plate.

75. A kit of parts according to Claim 73 or 74 wherein the detectable  
10 label conjugate comprises an anti-dog antibody.

76. A kit of parts according to Claim 73 or 74 wherein the detectable label conjugate comprises an enzyme.

15 77. A kit of parts according to Claim 76 further comprising a substrate for the enzyme.

78. A kit of parts according to any of Claims 73 to 77 further comprising a positive control sample that contains an anti-CRCV S protein antibody  
20 and/or an anti-CRCV HE protein antibody.

79. A method of passively immunising a dog against CRCV, comprising administering an antibody that reacts with CRCV to the dog.

80. A method according to Claim 79 wherein the antibody that reacts  
25 with CRCV comprises an anti-BCV antibody, an anti-HCV antibody, or an anti-CRCV antibody.

81. A method according to Claim 79 or 80 wherein the antibody that reacts with CRCV comprises an anti-S protein antibody or an anti-HE protein antibody.
82. A method according to any of Claims 79 to 81 wherein the antibody  
5 that reacts with CRCV comprises the anti-CRCV antibody according to Claim 20 or 23.
83. Use of an antibody that reacts with CRCV in the preparation of a medicament for passively immunising a dog against CRCV.
84. Use according to Claim 83 wherein the antibody that reacts with  
10 CRCV is an anti-BCV antibody, an anti-HCV antibody, or an anti-CRCV antibody.
85. Use according to Claim 83 or 84 wherein the antibody that reacts with CRCV is an anti-S protein antibody or an anti-HE protein antibody.
86. Use according to any of Claims 83 to 85 wherein the antibody that  
15 reacts with CRCV comprises the anti-CRCV antibody according to Claim 20 or 23.
87. A method of diagnosing CIRD the method comprising determining whether a dog has been exposed to CRCV according to the method defined in any one of Claims 24 to 36.